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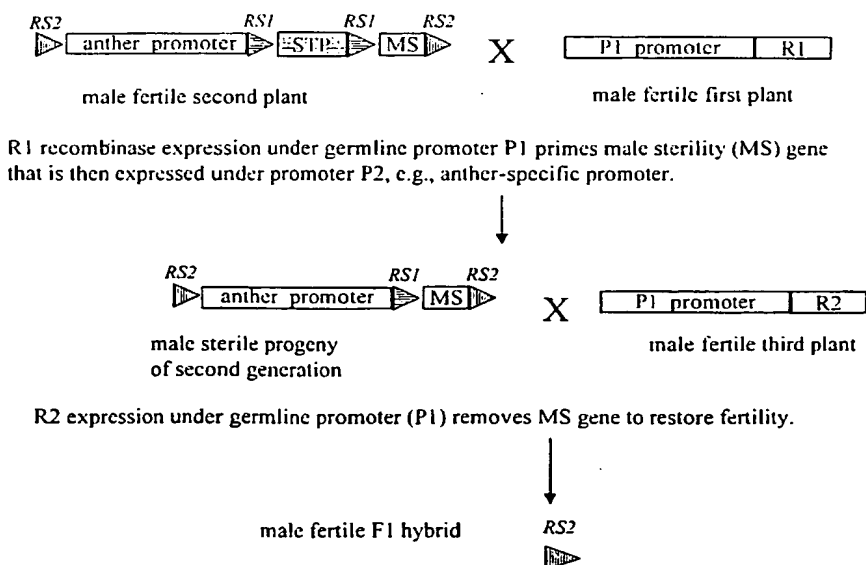
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR CONDITIONAL TRANSGENE EXPRESSION AND TRAIT REMOVAL IN PLANTS



(57) Abstract: This invention relates to constructs for the conditional or regulated expression or excision of transgenes in plants using site-specific recombinase systems. The constructs comprise a variety of constitutive, inducible, tissue specific or developmental stage-specific promoters operably linked to either a transgene or the elements of one or more site-specific recombinase system. By matching promoters, responsive to various inducers, plant tissues or plant developmental states with the recombinase systems, stop fragments and transgenes, virtually any trait may be expressed or excised at any plant development stage or in any plant generation.

WO 01/36595 A3

INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/90 C12N15/82 C12N9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, CHEM ABS Data, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ODELL J T ET AL: "SEED-SPECIFIC GENE ACTIVATION MEDIATED BY THE CRE/LOX SITE-SPECIFIC RECOMBINATION SYSTEM" PLANT PHYSIOLOGY, US, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 106, no. 2, 1 October 1994 (1994-10-01), pages 447-458, XP002912196 ISSN: 0032-0889	1,2, 4-31, 55-60,70
Y	page 454, paragraph 2; figure 1 the whole document	3-54, 61-70,73

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

18 June 2001

Date of mailing of the international search report

26.06.01

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/31600

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 11807 A (SNYDER JOHN R ;HODGES THOMAS K (US); LYZNIK LESZEK A (US); PURDUE) 11 March 1999 (1999-03-11) page 9, line 13 -page 10, line 16; figures	1,2, 4-31, 55-60,70
Y	the whole document	3-54, 61-70,73
	page 14, line 31 -page 15, line 11 ----	
X	WO 96 04393 A (US AGRICULTURE ;DELTA & PINE LAND CO (US)) 15 February 1996 (1996-02-15) page 10, line 17 -page 11, line 16; examples 6,9	1,2, 4-31, 55-60,70
Y	the whole document	3-54, 61-70,73

X	WO 91 09957 A (DU PONT) 11 July 1991 (1991-07-11)	1,2, 4-31, 55-60,70
Y	the whole document	3-54, 61-70,73

P,X	WO 00 17365 A (DU PONT ;YADAV NARENDRA S (US)) 30 March 2000 (2000-03-30)	1,2, 4-31, 55-60,70
Y	figure 3 the whole document	3-54, 61-70,73

X	MARIANI C ET AL: "INDUCTION OF MALE STERILITY IN PLANTS BY A CHIMAERIC RIBONUCLEASE GENE" NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 347, 25 October 1990 (1990-10-25), pages 737-741, XP002033710 ISSN: 0028-0836 the whole document	71,72
A		1-73

X	MCGONIGLE BRIAN ET AL: "Nuclear localization of the Arabidopsis APETALA3 and PISTILLATA homeotic gene products depends on their simultaneous expression." GENES & DEVELOPMENT, vol. 10, no. 14, 1996, pages 1812-1821, XP000996028 ISSN: 0890-9369 the whole document	71,72

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/31600

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>THEERAKULPISUT P ET AL: "ISOLATION AND DEVELOPMENTAL EXPRESSION OF BCP1 AN ANther-SPECIFIC CDNA CLONE IN BRASSICA-CAMPESTRIS" PLANT CELL, vol. 3, no. 10, 1991, pages 1073-1084, XP000986143 ISSN: 1040-4651 the whole document -----</p>	71,72

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/US 00/31600

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 2 (completely), 4 - 31 (partially),
55 - 60 (completely), 70 (partially)

relating to a combination of a recombinase construct and constructs containing sites responsive to said recombinase in order to activate or to remove a gene of interest.

2. Claims: 3 (completely), 4 - 31 (partially),
32 - 54 (completely), 61 - 69 (completely),
70 (partially), 73 (completely)

relating to a combination of a two different recombinase constructs and constructs containing sites responsive to said two recombinases in order to activate or to remove a genes of interest.

3. Claims: 71, 72

relating to a method for the germline-specific expression of a transgene.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claim 70 relates to a method employing an extremely large number of so-called recombinase elements. In fact the term "multiplicity of recombinase elements" does not limit their number in any way. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the elements claimed. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the combination of recombinase elements as disclosed in claim 1.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/31600

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9911807 A	11-03-1999	AU 9221098 A EP 1009843 A	22-03-1999 21-06-2000
WO 9604393 A	15-02-1996	US 5723765 A AU 696668 B AU 3205095 A BR 9508471 A CA 2196410 A EP 0775212 A JP 10503377 T TR 960448 A US 5977441 A US 5925808 A ZA 9506410 A	03-03-1998 17-09-1998 04-03-1996 28-10-1997 15-02-1996 28-05-1997 31-03-1998 21-07-1996 02-11-1999 20-07-1999 11-03-1996
WO 9109957 A	11-07-1991	AU 639059 B AU 6974791 A CA 2071943 A DE 69033142 D DE 69033142 T EP 0506763 A ES 2134767 T IL 96768 A US 5658772 A	15-07-1993 24-07-1991 23-06-1991 08-07-1999 23-12-1999 07-10-1992 16-10-1999 10-06-1997 19-08-1997
WO 0017365 A	30-03-2000	AU 731330 B AU 1122599 A AU 1441000 A BR 9815260 A EP 1025234 A HU 0004222 A PL 340297 A WO 9922003 A	29-03-2001 17-05-1999 10-04-2000 21-11-2000 09-08-2000 28-03-2001 29-01-2001 06-05-1999